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L3: Entry 1 of 4 File: USPT Sep 12, 2000

DOCUMENT-IDENTIFIER: US 6117635 A
TITLE: Nucleic acid amplification oligonucleotides with molecular energy transfer labels and methods based thereon

Drawing Description Paragraph Right (7):
FIG. 6 illustrates schematically the use of a FRET donor-acceptor-labeled hairpin primer in triamplification. In this embodiment of triamplification, unlike in PCR, a third oligonucleotide ("blocker") is ligated to the extended hairpin primer. The fluorescent signal is generated as a result of replication, however, as occurs in PCR.

Detailed Description Paragraph Right (78):
In another specific embodiment (FIG. 5), a universal hairpin primer is used, along with two selected linear primers, Primer 1 and Primer 2, to prime a PCR. In this case, the universal hairpin primer is incorporated into the amplification product and is not ligated to one of the two linear primer sequences. In this embodiment, the 3' sequence of the universal hairpin primer is identical to the 5' sequence of one of the pair of linear forward and reverse primers used in the amplification, and this 5' sequence (sequence "A" on Primer 2 in FIG. 5) must not be complementary to the target sequence.

Detailed Description Paragraph Right (95):
The hairpin primer is preferably labeled with a FRET donor-acceptor pair on its stem. During the first cycle of triamplification, the hairpin primer will be extended and ligated to the blocker. During the second cycle, the extended hairpin primer will become a template for the second primer. In the course of extension of the second primer, the hairpin will open, the quencher will be separated from the fluorophore and the donor will emit a fluorescence signal.

Detailed Description Paragraph Right (221):
This example presents experiments in which a universal hairpin primer was used, along with two selected linear primers, Primer 1 and "tailed" Primer 2, to prime a PCR amplification (see Section 5.2.1). The universal hairpin primer was incorporated into the amplification product and was not ligated to one of the two linear primer sequences. The 3' sequence of the universal hairpin primer was identical to the 5' sequence of one of the pair of linear forward and reverse primers used in the amplification, and this 5' sequence (sequence "A" on Primer 2 in FIG. 5) and was not complementary to the target sequence.

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L7: Entry 1 of 16 File: USPT May 15, 2001

DOCUMENT-IDENTIFIER: US 6232076 B1
TITLE: Stabilizer of dye sequencing products

Detailed Description Paragraph Right (2):
As used herein, the term "polymerase extension product" is a product of polymerase extension of a template nucleic acid. A preferred polymerase extension product is a deoxyribonucleic acid (DNA), but ribonucleic acid (RNA) extension products are also applicable. The nucleotides in the polymerase extension product can be the common nucleotides which naturally occur in genomic DNA and RNA, i.e., the monophosphates of deoxyadenosine, deoxyguanosine, deoxycytidine, deoxythymidine, adenosine, guanosine, cytidine, and uridine. The polymerase extension product can also include other nucleotides which may be useful replacements for any of the above. Non-limiting examples are deoxyinosine monophosphate and 7-deaza-deoxyguanosine monophosphate. As is well known, nucleotides which are particularly useful for polymerase extension products used in sequencing procedures are 3' terminal dideoxyribonucleotides such as dideoxyguanosine, dideoxyadenine, dideoxycytidine and dideoxythymidine. The skilled artisan can readily further identify which nucleotides are useful for any particular polymerase extension product application.

L6: Entry 7 of 8 File: USPT Dec 29, 1992

DOCUMENT-IDENTIFIER: US 5174962 A
TITLE: Apparatus for determining DNA sequences by mass spectrometry

Detailed Description Paragraph Right (1):
This invention relates to methods, reagents, apparatus and intermediates involved in the determination of natural or artificially made ("recombinant") DNA sequences and fragments thereof. This invention seeks to eliminate numerous deficiencies in the prior art by embodying greater convenience, less chromatographic ambiguity, greater sensitivity and safer handling than existing procedures. In particular, this invention involves the determination of DNA sequences using a combination of chain termination DNA sequencing techniques and mass spectroscopy. Thus, in a typical chain terminating DNA sequencing determination such as taught by Sanger, et al., Proc. Natl. Acad. Sci. USA, 74, 5463, (1977) involving a DNA primer, deoxynucleotidetriphosphates, dideoxynucleotidetriphosphates in the presence of a DNA polymerase, such as Kenow fragment, are used to determine the DNA sequence. However, in embodiments of the present invention the DNA primer, the deoxynucleotides or the dideoxynucleotides are labeled with isotopes detectable by mass spectrometry to determine the DNA sequence. For example, if the dideoxynucleotides (A, G, C, T) triphosphates, abbreviated as ddATP, ddGTP, ddCTP and ddTTP respectively, are labeled with isotopes of different masses respectively, and chain terminated fragments corresponding to those fragments are separated and analyzed by mass spectrometry, a direct component of each dideoxynucleotide component of the chain terminated DNA sequence is converted to a more convenient species for mass spectrometry determination, i.e. sulfur isotopes are oxidized to sulfur dioxide. If the DNA primer or deoxynucleotides are labeled, reactions between specifically labeled deoxynucleotides must be first carried out in the presence of a specific dideoxynucleotide. This is necessary so that a specific label is associated with a specific chain terminated DNA sequence. Once the individual reactions are conducted, the chain terminated DNA sequences can be mixed, separated, and analyzed by mass spectrometry because there will then be a specific relationship between a specific isotope and the terminal dideoxynucleotide. This invention is much more sensitive than existing systems and therefore is especially useful in determining the sequence of small quantities of DNA which are contaminants in products resulting from fermentation and other biotechnology related processes, i.e. for "screening" applications. The invention also includes reagents and analytic instruments for carrying out the above methods as well as intermediate mixtures of chain terminated DNA sequences produced while carrying out the methods of the present invention.

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L9	mass near0 match$	217	L9
L8	mass near0 match$ near0 nucle$	0	L8
L7	deoxyinosine same (advantag$ or useful$)	16	L7
L6	L1 same dideoxy$	8	L6
L5	L1 same deoxyinosine	0	L5
L4	L2 same deoxyinosine	0	L4
L3	L2 same dideoxy$	0	L3
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L9	L8 same first	51	L9
L8	L7 same 5 same cleav$	56	L8
L7	L6 same 3	629	L7
L6	(primer near0 second) same (nucleic or DNA or oligo$)	1440	L6
L5	L1 same (advantag$ or useful$)	7	L5
L4	L3 same (advantag$ or useful$)	0	L4
L3	L1 same ligat$	4	L3
L2	L1 (p)ligat$	1050948	L2
L1	hairpin near0 primer$	24	L1

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L5: Entry 1 of 7 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277607 B1
TITLE: High specificity primers, amplification methods and kits

Detailed Description Paragraph Right (6):
Hairpin primers of the present invention can contain deoxyribonucleotides, ribonucleotides, peptide nucleic acids (PNA), other modified nucleotides, or combinations of these. Modified nucleotides may include, for example, 2'-O-methylribonucleotides or nitropyrole-based nucleotides. Modified internucleotide linkages may also be included, for example phosphorothioates. Using modified nucleotides in the 5' arm provides a way to adjust the strength of the stem. Other advantages of using such modifications for a particular application will be apparent to persons familiar with the art. In particular, hairpin primers according to this invention that are constructed from modified nucleotides may form stronger hybrids than if the primers were constructed from deoxyribonucleotides, thus enabling structured target sequences (such as those that occur in messenger RNAs) to be more easily accessed.

Detailed Description Paragraph Right (8):
Hairpin primers of the present invention permit monitoring of amplification reactions by fluorescence. They can be labeled with interactive fluorescent moieties, and with other labels, in such a manner that free primers permit the interaction of the label moieties, whereas the labels of primers that are incorporated into double-stranded amplicons do not so interact. One of the interactions that are useful in detection is fluorescence energy transfer, which causes the fluorescence of free primers to be quenched and the fluorescence of those that are incorporated into amplicons not to be quenched. As a result, the course of the amplification reactions can be monitored by measuring the fluorescence. Particularly useful is the interaction of quenching by touching, wherein a non-fluorescent quenching molecule such as DABCYL, DABMI, DABSYL or Methyl Red is used to prevent a fluorophore from fluorescing, rendering free-floating primers dark. Because (a) hairpin primers according to this invention are so specific that they are extremely unlikely to generate false amplicons, and (b) their particular hairpin shape markedly reduces the probability that they will interact with each other to form primer-dimers, the generation of a fluorescent signal in a reaction that utilizes hairpin primers according to this invention is an excellent indication that the intended amplicons have been synthesized. Furthermore, the generation of a fluorescent signal during real-time detection of the amplification products allows accurate quantitation of the initial number of target sequences in a sample. This represents an improvement over the current art. In the current art, either hybridization probes have to be used to monitor the amplification reactions, or when other methods of amplicon labeling are used, these methods are not sufficiently specific to discriminate false amplicons from the intended amplicons (Nazarenko et al., 1997; Wittwer et al., 1997).

Detailed Description Paragraph Right (9):
The advantages of using hairpin primers according to this invention are unexpected. It has been a common belief that the presence of a hairpin in the priming region of a primer, deleteriously diminishes the ability of the primer to prime. Examples of such teachings can be found in computer programs that are used to design primers for polymerase chain reactions, where the algorithms reject putative primer sequences that possess self-complementary (hairpin-forming) sequences (for example, Haas et

al., 1998; and Rychlik et al., 1989).

Detailed Description Paragraph Right (11):
Hairpin primers of this invention are useful in a number of nucleic acid amplification processes that employ primers, including polymerase chain reactions (PCR), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), transcription-mediated amplification (TMA), and rolling-circle amplification (RCA). The high specificity and consequent low mis-priming significantly and detectably delays signal from sequences other than perfectly complementary targets. In thermal cycling amplifications, this delay is manifested in a later threshold cycle: in isothermal amplifications, in a later time for detectable signal to arise.

Detailed Description Paragraph Right (28):
The design of the first and second hairpin primers is illustrative of two design points. For the first hairpin primer we noted that a loop eight nucleotides long and a stem six nucleotides long could be obtained by adding only three nucleotides to the 5' terminus of the first conventional primer. That was possible in this particular instance, because of fortuitous complementarily. Note that three nucleotides in the 5' arm are there nucleotides at the 5' end of the first conventional primer. For the second hairpin primer the 5' arm includes six added nucleotides. However, in this instance note that the loop, thirteen nucleotides long, is outside the preferred range of 5-12 nucleotides. In this instance we were not attempting to discriminate against a single nucleotide substitution. Four polymerase chain reactions were performed, two containing the conventional primers, and two containing the hairpin primers. One of the reactions in each pair was initiated with target template DNA, whereas the other reaction in the pair did not contain any template DNA. The progress of these reactions was monitored using the fluorescent intercalating dye, SYBR Green, to label any amplicons that were generated in the course of the amplification reactions. The changes in fluorescence intensity that were observed during the course of the amplification reactions is shown in FIG. 7. The results show that the reaction that contained the first and second conventional primers and 20,000 template molecules, curve 71, became positive after 16 thermal cycles had been completed, and the reaction that contained those conventional primers and no template DNA, curve 73, became positive after 29 thermal cycles had been completed, indicating that false amplicons were generated in the course of the reaction. A subsequent analysis of these amplicons by polyacrylamide gel electrophoresis showed that legitimate amplicons were produced in the first reaction, whereas false amplicons of an unexpected size were produced in the second reaction. We believe the false amplicons were primer-dimers. However, when the first and second hairpin primers were used in place of the conventional primers, the reaction initiated with 20,000 target template molecules, curve 72, became positive after 17 thermal cycles had been completed, but the reaction that did not contain any template molecules, curve 74, never became positive. Subsequent analysis of the amplicons by polyacrylamide gel electrophoresis confirmed that the first reaction generated the expected amplicons, and the second reaction did not generate any amplicons. These results confirm that hairpin primers of the present invention are useful in solving the problem of false amplicon synthesis during amplification reactions. The reason that hairpin primers suppress the synthesis of false amplicons is that only the sequence in the loop is available for initiating the primer-template hybrid, and the presence of a hybridization sequence in a hairpin loop renders the interaction between the primer and the target nucleic acid much more specific than the interaction that occurs when a conventional linear primer hybridizes to a target nucleic acid. Moreover, the structure of hairpin primers is such that the sequences that are present in the arms of the primer do not participate in the initial hybridization of the primer to the target nucleic acid. Consequently, the binding of one primer to another during an amplification reaction (which can create undesirable amplifiable primer-dimers when conventional linear primers are used) is much less likely to occur when hairpin primers are used.